Highly Controllable Electroporation and Applications Thereof

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Related Applications

This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 60/439,387 filed on January 10, 2003, which is herein incorporated by reference

Background

The membrane of a cell serves the vital function of partitioning the molecular contents from its external environment. The membranes are largely composed of amphiphilic lipids, which self-assemble into highly insulating structures and thus present a large energy barrier to trans-membrane ionic transport.

However, the lipid matrix can be disrupted by a strong external electric field leading to an increase in trans-membrane conductivity and diffusive permeability, a well-known phenomenon known as electroporation. These effects are the result of formation of aqueous pores in the membrane. More particularly, electroporation process involve permeation of cell membranes upon application of short duration electric field pulses, traditionally between relatively large plate electrodes (Neumann, et al., Bioelectrochem Bioenerg 48, 3-16 (1999); Ho, et al., Crit Rev Biotechnol 16, 349-62 (1996)).

For example, Figures 2A and 2B show a conventional electroporation system 20, whereby impression of an electric field (e.g., shown by closing a circuit 22) creates random pores 26 in a cell membrane 24. The distribution of such pores, in terms of size and number only, is determined by the strength and duration of the applied electric field. The stronger and the longer the electric field is applied, the more numerous and larger the pores are. However, the exact location of such pores cannot be controlled, and thus the final distribution of pores is somewhat random. Researchers lose

control over where compounds are introduced into the intracellular matrix, an oft-important ingredient in biochemical pathways. Thus researchers often have to rely on the cell's own natural mechanisms, a far slower and difficult process to utilize.

Electroporation is used for introducing macromolecules, including DNA, RNA, dyes, proteins and various chemical agents, into cells. Large external electric fields induce high trans-membrane potentials leading to the formation of pores (e.g., having diameters in the range of 20-120 nm). During the application of the electric pulse, charged macromolecules, including DNA, are actively transported by electrophoresis across the cell membrane through these pores (Neumann, et al., Biophys J 712 868-77 (1996)). Uncharged molecules may also enter through the pores by passive diffusion. Upon pulse termination, pores reseal over hundreds of milliseconds as measured by recovery of normal membrane conductance values (Ho, 1996, supra).

This procedure is often used in laboratory settings to inject chemical and biological compounds into a cell, avoiding the reliance on the cell's own protein receptors and trans-membrane channels for transport across the cell membrane. This allows researchers to easily study the biological affect of compounds, be it a potentially life-saving cancer drug or a deadly biological toxin. However, current electroporation techniques are limited.

Therefore, it would be desirable to provide a method and system to overcome these and other limitations of conventional electroporation.

SUMMARY

The above-discussed and other problems and deficiencies of the prior art are overcome or alleviated by the several methods and apparatus of the present invention for controllable electroporation. The controllable electroporation system and method allows control over the size,

the number, the location, and the distribution of aqueous pores, thus increasing flexibility of use. The herein described system and method for controllable electroporation generally employs at least two actuating sub-systems and sub-processes. One sub-system and sub-process employs a relatively broad effect in order to weaken the membrane, a broad effect sub-system. Another sub-system and sub-process employs a relatively narrow effect in order to localize the position of the pore in the membrane, a narrow effect sub-system.

The above-discussed and other features and advantages of the present invention will be appreciated and understood by those skilled in the art from the following detailed description and drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B show operation of the controllable electroporation system described herein;

Figures 2A and 2B show operation of conventional electroporation systems;

Figures 3A-3D show a general embodiment of a cell injection system;

Figure 4A-4B shows an example of a cell injection system;

Figure 5A-5B shows an example of a cell injection system for an array of cells;

Figure 6 shows a separation device using multi-phospholipid layers and electroporation;

Figure 7 shows a separation device using multiple layers, electroporation and a microfluid array;

Figure 8 shows a separation device using a single layer capapble of having different pores at different locations; and

Figure 9 shows an exemplary electrode grid that may be used in various embodiments described herein.

DETAILED DESCRIPTION

Herein described is an electroporation system and method providing control over the size, the number, the location, and the distribution of aqueous pores, thus increasing flexibility of use. Referring generally to Figures 1A and 1B, the herein described system 10 and method for controllable electroporation of a membrane 12 generally employs at least two actuating sub-systems and sub-processes. Figure 1A shows a portion of a membrane 12, and Figure 1B shows the controllable electroporation system 10 described herein.

Note that in general, one of the actuating sub-systems alone will not suffice to open or create a pore 14 in the membrane – both actuating sub-systems are employed, thereby functioning in a similar manner as a logical "and" gate. A broad effect sub-system 16 employs a relatively broad effect in order to weaken the membrane, and the narrow effect sub-system 18 employs a relatively narrow effect in order to localize the position of the pore 14 in the membrane 12. Employing both the broad effect sub-system 16 and the narrow effect sub-system 18 enables highly localized and controlled electroporation and hence opening of pore 14.

Therefore, for example as compared to conventional electroporation processes of cellular membranes, described in the Background and with respect to Figures 2A and 2B, in addition to the applied electric field of conventional electroporation technology, the herein disclosed electroporation system and method employs a narrow effect sub-system 18 directed at a specific location on the cellular membrane, enabling positional control over the pore 14. The narrow effect sub-system 18 will excite the phospholipid molecules, thus reducing the amount of energy required

from the broad effect sub-system 16 to create the aqueous pores. Thus, for example, a very weak electric field can be applied to a system, in which typically, electroporation would not occur. However, this weak electric field can open aqueous pores in places of the cellular membrane already excited by the laser beam.

The broad effect sub-system or sub-process 16 may be selected from any suitable membrane weakening systems and/or processes. Such weakening systems and/or processes may be selected from the group consisting of electric fields (in certain preferred embodiments uniform electric fields), microwave energy, other electromagnetic radiation, relatively low energy laser beams (i.e., lower energy than that required to commence random electroporation), or any combination comprising at least one of the foregoing weakening systems and/or processes. The energy magnitude of the broad effect sub-system or process 16 is generally lower than the energy magnitude of conventional electroporation systems whereby random pore opening occur. Further, the area (e.g., cross-sectional area) of the weakening systems and/or processes 16 generally encompasses an area larger than the desired pore size. In certain embodiments, this area encompasses the entire cell membrane or an array of cell membranes. In other embodiments, this area is a region of a membrane.

The narrow effect sub-system or sub-process 18 may be selected from any suitable membrane pore position localization systems and/or processes. Such position localization systems and/or processes may be selected from the group consisting of laser beams, electrode tips, or any combination comprising at least one of the foregoing position localization systems and/or processes. The area (e.g., cross-sectional area) of the position localization systems and/or processes 18 is generally narrow, e.g., corresponding to the desired dimensions of the pore opening. Thus, for example, controlled pore openings having of sub-micron or nanometer (e.g., 1-100 nm) magnitude

are enabled, since existing and developing laser and electrode tip technologies are capable of such sub-micron-scale and nano-scale dimensions.

Applications

Cell Injection

The herein described controllable electroporation system and process may be used to inject macromolecules, including DNA, RNA, dyes, proteins and various chemical agents, in a controlled manner. Without intending to limit the applications of the present controllable electroporation system, Figures 3A-5B show various embodiments of cell pore opening systems employing the controllable electroporation system.

Referring now to Figures 3A-3D, a system 30 is shown for controllable injecting macromolecules into a cell. Figure 3A depicts the system 30 including a mechanism 32 for holding a cell 24. Mechanism 32 is a suitable microrobotic device including associated microsystems as are generally known in the biotechnology arts. Such as device 32 preferably is capable of holding individual cells or controlled groups of cells. Further, mechanism 32 may also be used to obtain biological, electrical, optical, or other data from the cell 24.

Referring now to Figure 3B, the system 30 is shown including the mechanism 32 holding the cell 24, and a controllable electroporation system including the broad effect sub-system 16 and the narrow effect sub-system 18, whereby the narrow effect sub-system 18 is focused at a location on the cell 24 to induce opening of a pore 34.

Referring now to Figure 3C, a macromolecule 38 is introduced, for example, via a nano-nozzle or other suitable injection device 36. When the narrow effect sub-system 18 and/or the broad effect sub-system 16 is removed, the pore will close, resulting in cell 24' having macromolecule 38 therein.

Figures 4A and 4B show one embodiment of a controllable electroporation system 40 for introducing controllably opening a pore 34 in a cell 24, e.g., for introduction of macromolecules as described above. The system 40 includes a broad effect sub-system in the form of an electric field producing apparatus 42, 44, 46, and a laser beam 48 from a suitable source (not shown). The electric field producing apparatus is in the form of an electrode plate 42 coupled to a switchable (via a switch 44) voltage source 46. As shown, the laser beam may be focused, and the electric field applied to active the pore opening mechanism, akin to a logical "and" circuit as described above.

With the system and method described with respect to Figures 3A-3D and 4A-4B, researchers could only expose a few cells in a tissue construct to a biological compound and observe how the signal is propagated to its neighboring cells. Alternatively, researchers could study if asymmetric cells such as neurons and gastrointestinal mucosa cells react differently to compounds injected at different places.

Referring now to Figures 5A and 5B, a system 50 is shown that operates similar to that of Figures 3A-3D or 4A-4B in conjunction with an array 52 of cells 24. When the broad effect sub-system 16 and the narrow effect sub-system 18 are operated, pores 34 are formed in the cells 24. Such pores may be used for selective introduction of macromolecules into the cells 24.

Separation Device

Referring now to Figures 6-10, various embodiments of filtration/separation devices are provided using the controllable electroporation system herein.

Figure 6 depicted one embodiment of a system 60, e.g., a molecular sieve. System 60 includes plural membrane layers 62, e.g., phospholipid bilayers. Each membrane layer 62 may be subject to a narrow effect sub-system during application of the broad effect sub-system,

alternatively the location of the pores 64 may be predetermined upon assembly and or manufacture, e.g., with suitable micro- or nano-defects, or may be identical whereby different voltage levels at each layer determined the pore size. Application of different voltages (e.g. V1, V2 and V3) at each layer creates a filter gradient from large pores 64 to small pores 64, allowing passage of molecules 66 through suitable layers.

Using a phospholipid bilayer, which is extremely cheap to manufacture, the same filter 60 can be used repeatedly and adapted for any size requirements using electroporation and carefully controlling the electric field that is applied. Instead of depending on multiple filters, a single filter could be used and configured for any situation.

Referring to Figure 7, a system 70 is shown with a molecular sieve functioning similar to that of Figure 6 associated with a biochip array 72 having channels 74 therein. Channels 74 may serve to collect macromolecules and molecules at each level based on size. Further, channels 74 may incorporate or be associated with a gradient system, e.g., pressure, thermal, electrical, or other gradient to induce macromolecules and molecules toward the array 72. Array 72 may be any suitable microfluidic or nanofluidic device. For example, methods of manufacturing such devices are described in Reveo Inc. PCT Application No. PCT/US03/37304 filed November 30, 2003 entitled "Three Dimensional Device Assembly and Production Methods Thereof", which is incorporated by reference herein.

Figure 8 shows another example dynamic filtration device, wherein an array of lasers provides positional control over the pore openings. Figure 80 shows a filtration system 80 including a membrane layer 82 associated with a broad effect energy sub-system 16 and a narrow effect sub-system 18. For example, the narrow effect sub-system 18 may be generated with a laser array 88. Alternatively, instead of an array of lasers 88, a beam steering device may be

incorporated, allowing use of only one laser source. When a laser is activated from the array associated with a certain position on the membrane 82, a pore 84 will open. The size of the pores may be controlled by predetermined membrane characteristics, area or magnitude of the narrow effect energy sub-system, or magnitude of the broad effect energy sub-system.

Cells, proteins, enzymes, DNA molecules, RNA molecules, and other macromolecules or molecules may be collected via an array of containers 86, e.g., on a suitable microfluidic device. Thus, separation device 80 may be made extremely compact and highly flexible for any purpose.

Figure 9 shows an example of an electrode suitable for providing the broad effect energy sub-system in various embodiments shown herein. By providing electrodes in a grid pattern, a suitable field generating system may be provided to allow access for various purposes including the narrow effect sub-system, macromolecule introduction, filtration, or any other purpose.

In addition to filtering based on size, the aforementioned separation devices may also separate on the basis of ionic charge, since the applied voltage will drive only one type of ions across the membrane.

While preferred embodiments have been shown and described, various modifications and substitutions may be made thereto without departing from the spirit and scope of the invention. Accordingly, it is to be understood that the present invention has been described by way of illustrations and not limitation.